

THE STRUCTURE AND FUNCTION OF CENTRIOLAR SATELLITES AND  
PERICENTRIOLAR PROCESSES IN CNIDARIAN SPERM

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INTRODUCTION

During spermiogenesis cnidarian sperm centrioles associate with a number of accessory structures including microtubular nucleating satellites and pericentriolar processes. These centriolar specializations are particularly well developed in cnidarian sperm and appear to be highly involved in sperm development and function.

Microtubular nucleating satellites are thought to be involved in the assembly and disassembly of microtubules (De The, 1964; Boisson et al., 1969 and Tilney and Goddard, 1970). The microtubules that often radiate from satellites have been implicated in cytoskeletal phenomena seen in a number of cell types (Porter, 1966; Gibbins et al., 1969 and Tilney and Gibbins, 1969). With respect to vertebrate sperm they are involved in nuclear shaping and formation of the manchette (Burgos and Fawcett, 1955; Tilney and Gibbins, 1969 and Rattner, 1972). The function of cytoplasmic microtubules during spermiogenesis in invertebrate sperm is unclear.

Pericentriolar processes are unusual structures associated with the centrioles of many animal cells, especially the distal centrioles of invertebrate sperm including the Cnidaria (Dewel and Clark, 1972; Summers, 1972; Hinsch and Clark, 1973 and Hinsch, 1974). In many instances pericentriolar processes have been confused with microtubular nucleating satellites. Satellites associate with the anterior half of sperm centrioles in early spermiogenesis. As spermiogenesis advances pericentriolar processes appear at the posterior end of the distal centriole existing simultaneously with the anterior satellites for a short period. This period of coexistence, typical of most cnidarian sperm centrioles, has undoubtedly contributed to

much of the confusion surrounding satellites and pericentriolar processes.

In the present study we compared the structure and function of satellites and pericentriolar processes in the hydrozoan, Hydractinia echinata. Our preliminary studies have included a careful ultrastructural examination of the morphological relationships between sperm centrioles and their associated specializations in both thin section and whole mounts of disrupted sperm.

## METHODS

Male colonies of H. echinata were collected from shells occupied by the hermit crab Pagurus sp. in Galveston Bay, Texas or Woods Hole, Massachusetts. Synchronous gonadal development was induced by exposure to continuous photoperiods of one to seven days (Ballard, 1942). Sperm or spermatids of uniform maturity were obtained from excised gonophores with several strokes of a loose teflon homogenizer.

Isolated early spermatids or mature sperm were washed (3x) in sterile seawater by sedimentation at 1000 xg to remove gonophore cells and debris released as the gonophores were broken. Clean preparations were sedimented at 5000 xg, resuspended in a hypotonic saline and allowed to swell for 10 minutes. The swollen sperm were disrupted by brief ultrasonication. These whole cell homogenates were applied in a drop-wise fashion to 0.4% formvar coated, carbon reinforced grids. The excess fluid was immediately drawn off with filter paper. Whole mount preparations were stained by the addition of a drop of 0.2% aqueous uranyl acetate which was allowed to stand for 60 seconds before being drawn off with filter paper.

Tissue for thin section electron microscopy was fixed in a glutaraldehyde-paraformaldehyde mixture (Karnovsky, 1965) buffered in 0.1M sodium phosphate (pH 7.2). Following a buffer wash, the tissue was post-fixed in 1.0% osmium tetroxide buffered as above, rapidly dehydrated in an acetone series and embedded in low viscosity epoxy resin (Spurr, 1969). Both thin section and whole mounts of disrupted sperm were examined with an AEI EM6B or Hitachi HS-8 electron microscope.

## RESULTS

Microtubular nucleating satellites are the first centriolar specializations to associate with Hydractinia sperm centrioles during spermiogenesis. These satellites appear as spheres of electron dense material that seem to diffuse into the cytoplasm leaving no well defined margin. At times a distinct connection exists

between a satellite and the outer surface of the centriolar matrix. Satellites are attached to the matrix by a stalk of electron dense material (Fig. 1). The stalk tapers, being widest at the point of matrix attachment (80 to 100 m $\mu$ ) and narrowest at the point of satellite attachment (40 to 60 m $\mu$ ). Individual variations in size and the diffuse periphery of the satellites do not allow precise measurement; however, the approximate value of 100 m $\mu$  is consistent with previous reports (Tilney and Goddard, 1970).

Satellites that surround and associate with Hydractinia sperm centrioles are the site of extensive microtubular radiation which persists through most of spermiogenesis (Fig. 2). These microtubules concentrate in the developing spermatid midpiece region. Microtubules extend from the distal and proximal centrioles to the periphery of the cell and are found anchored on the plasma membrane (Fig. 3). Microtubules also run between the sperm plasma membrane and the mitochondria forming a band that girdles the circumference of the sperm midpiece (Fig. 4).

Pericentriolar processes associate with the distal centrioles of Hydractinia sperm about mid-way through spermiogenesis. The process complex is composed of nine pericentriolar processes which emanate from the centriolar matrix between the triplets (Fig. 5). Each of the nine members is composed of a primary process which extends out from the matrix for 200 m $\mu$  terminating in a thickened tip and three secondary processes which radiate from that tip. The secondary processes extend into the cytoplasm between the mitochondria and plasma membrane where they terminate in close apposition to the membrane (Fig. 6). Inter-primary processes are additional components observed in thin sections. The structures interconnect adjacent primary processes by extending from the base of one primary process, near the point of matrix attachment, to a point near the thickened tip of the next primary process. Interprimary processes parallel the plane of the centriolar triplet blades while the primary processes are perpendicular to the triplet blades.

Disrupted whole mounts of spermatids from the early stages of spermiogenesis, before pericentriolar process formation is complete, yield preparations which contain centrioles with attached satellites (Fig. 7). The satellites in whole mount preparations demonstrate a considerably different morphology from those seen in thin section. They are generally ovoid in shape rather than spherical. Their peripheral margins are well defined and the outer edge of the ellipse (distal to the centriole) is often drawn to a point. Satellites seen in whole mount demonstrate a core and cortex. Often the core has a crystalloid appearance in these air dried preparations (Fig. 7). The tapered stalk seen in thin sections is readily apparent. Satellites viewed in whole mount preparations are no longer associated with the numerous microtubules seen in thin section.

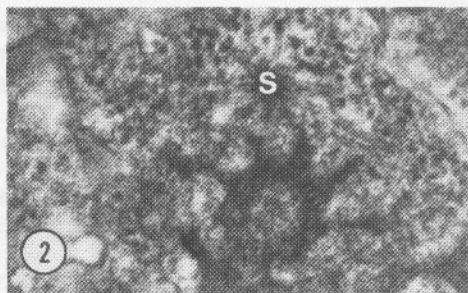
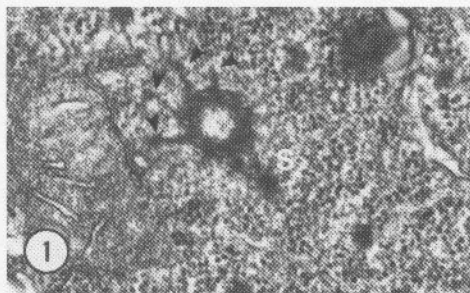


Figure 1. Tangential section through a distal centriole reveals an anterior satellite (S) attached to the centriole by a tapered stalk and the posterior pericentriolar processes (arrows) which are starting of form. X38,000.

Figure 2. Distal centriole with closely positioned satellite (S) which is the nucleation site for numerous microtubules. Pericentriolar processes are also apparent. X54,000.

Figure 3. Longitudinal section of a late spermatid showing several microtubules which run from the proximal centriole to the posterior plasma membrane. Pericentriolar processes can be seen emanating from the distal centriole. X32,000.

Figure 4. Late spermatid showing microtubules that run in a band between the plasma membrane and mitochondria of the midpiece (arrows). X45,000.

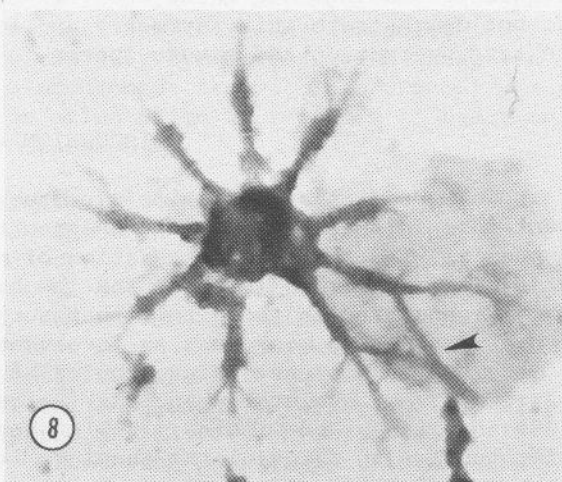
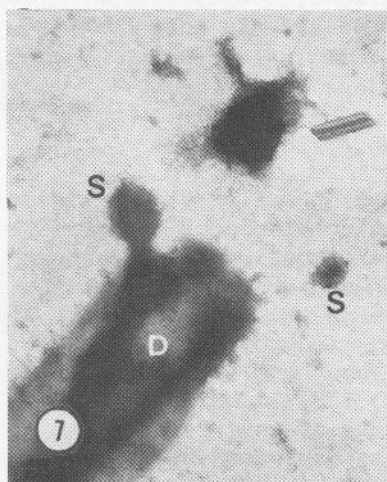
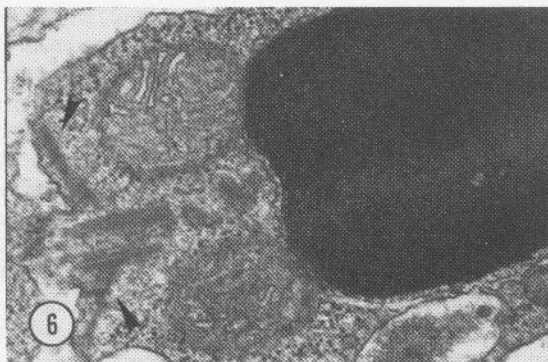
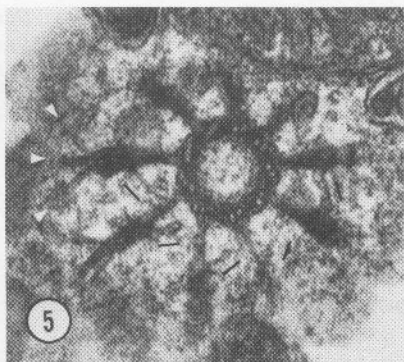


Figure 5. Cross section passing through a distal centriole at the level of the pericentriolar processes. Nine primary processes can be seen emanating from the centriolar matrix. The primary processes terminate in thickened tips and three secondary processes emanate from these tips (arrows). Interprimary processes which connect adjacent primary processes are marked on the micrograph by lines drawn parallel to them. X50,000.

Figure 6. Longitudinal section passing through the mid-plane of a late spermatid. The pericentriolar processes (arrows) emanate from the posterior end of the distal centriole and extend out into the cytoplasm between the plasma membrane and mitochondria. X25,000.

Figure 7. Whole mount of free distal centriole (D) from early spermatid. Two satellites (S) can be seen. One attached to the anterior portion of the centriole by the tapered stalk, and the other lying close to the centriole. X56,000.

Figure 8. Whole mount of a free distal centriole from a mature sperm. The asymmetry of the complex is demonstrated by the two long secondary processes. The point of anastomosis between these processes is marked by an arrow. X27,000.

Pericentriolar processes liberated from the disrupted sperm are well preserved (Fig. 8). They retain their primary processes ending in thickened tips and secondary processes which also appear to terminate in thickened tips. In whole mount the process complex appears asymmetrical, an aspect poorly discernible in thin section because of the angle of process projection. This asymmetry is demonstrated by the secondary extensions of two adjacent primary processes. These are much longer than those extending from the other seven (Fig. 8). Previous thin section observations of Hydractinia sperm (Hinsch and Clark, 1973) suggested that fusion took place between the outermost members of each set of three secondary processes. In whole mount, it is clear that this anastomosis takes place only between the long and not between the seven short secondary processes. Centriolar complexes viewed in whole mount and thin sections from early spermatids do not demonstrate this asymmetry and fusion. It is only observed in late spermatids and mature sperm.

#### DISCUSSION

In Hydractinia spermatids microtubules appear to play a significant role in the morphological changes taking place during spermiogenesis. The intimate association of microtubules with the spermatid plasma membrane (Fig. 3) and the high degree of organization they demonstrate in the girdle that surrounds the midpiece (Fig. 4) suggest extensive microtubular involvement in cytoskeletal shaping of the sperm midpiece. Microtubules have been implicated in a wide variety of cytoskeletal phenomena (Porter, 1966; Gibbins et al., 1969 and Tilney and Gibbins, 1969). Because of this involvement of microtubules in cytoskeletal shaping, it seems reasonable to assume that centrioles carrying microtubular nucleating satellites may play a regulative role in the structural changes taking place during sperm differentiation.

Although many researchers have failed to make a distinction between pericentriolar processes and satellites, Dewel and Clark (1972) have clearly distinguished these as separate structures. The data presented in this study confirm their view that pericentriolar processes are distinct from satellites in both form and location. The processes are situated at the posterior end of the distal centriole while satellites are more anteriorly located. Satellites are ovoid or spherically shaped structures that have a somewhat amorphous appearance in thin section. Satellites are apparently centers for the nucleation of microtubules which are found associated with them the entire time they are present in the developing spermatid. Pericentriolar processes are long, branching and striated structures always numbering nine, one for each centriolar triplet. They have not been observed to act as centers for the convergence of microtubules or to be involved in microtubular nucleation.

Since pericentriolar processes are distinct from satellites, the question remains, what is their function? Previous investigators have suggested two possible roles; one structural, *i.e.* an anchoring mechanism (Szollosi, 1964), and another contractile involved in locomotor activity (Summers, 1972; Dewel and Clark, 1972 and Kleve and Clark, 1974). The contractile role is very appealing when one considers the chemotactic responses demonstrated by many sperm (for review see Miller, 1973). The most obvious and clearly demonstrated chemotactic sperm behavior is seen in various cnidarian species including *Hydractinia* (Miller, 1974 and Miller and Tseng, 1974). It is interesting that sperm of species with elaborate pericentriolar processes often demonstrate extensive chemotactic behavior. Such responses necessitate directional selectivity by sperm. Response to chemotactic stimuli by alterations in the asymmetry of the sperm midpiece would provide the "rudder" necessary for directional selectivity. *Hydractinia* sperm are radially symmetrical with exception of the asymmetric pericentriolar process array. This asymmetry may in some way give the sperm the orientation it requires to react to and move toward released chemotactins.

When sperm react chemotactically they are doing so as a one-cell sensory unit. In such an instance the sperm flagellum may well act in a manner similar to sensory cilia. It is interesting to note that a number of investigators have observed extensive pericentriolar process complexes associated with the basal bodies of a wide variety of sensory cilia. A partial list would include sensory cilia of the vertebrate inner ear and lateral line organs (Flock and Duvall, 1965; Wesall *et al.*, 1965 and Flock and Jorgenson, 1974); olfactory cilia (Reese, 1965); and photoreceptive cilia (Tokuyosu and Yamada, 1959 and Horridge, 1964). Sensory cilia with complex centriolar structures are also found in neurosensory cells of hydra (David, 1969 and 1974), cnidocils and distal flagella of cnidarian nematocytes (Westfall, 1965 and 1970) as well as the pedicellaria of echinoderms (Cobb, 1967). The role pericentriolar processes play in sensory cilia function is evasive. One cannot readily see the need for a contractile unit at the base of seemingly non-locomotor cilia unless they facilitate the orientation of the sensory cilia toward a stimulus. Their frequent association with sensory cilia would imply a functional connection. In the case of cnidarian sperm pericentriolar processes may serve in both a sensory and locomotor capacity.

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#### SUMMARY

Microtubular nucleating satellites have been distinguished from pericentriolar processes in structure, location, chronology of appearance and function. Satellites associate with the anterior

half of Hydractinia sperm centrioles early in spermiogenesis, are spherical in shape and involved in the assembly of microtubules responsible for spermatid shaping. Pericentriolar processes associate with the posterior end of distal centrioles late in spermiogenesis. They are long trifurcated structures always numbering nine, one for each centriolar triplet blade. Pericentriolar processes never associate with microtubules; however, they may be contractile and involved with the chemotactic behavior of sperm.

#### REFERENCES

- Ballard, W. W. 1942. The mechanism for synchronous spawning in Hydractinia and Pennaria. Biol. Bull. 82(3):329.
- Boisson, C., X. Mattei and C. Mattei. 1969. Mise en place et evolution du complexe centriolaire au course de la spermiogenèse d'Upeneus prayensis C. V. (Poisson Mullidae). J. Microscopie. 8:103.
- Burgos, M. H. and D. W. Fawcett. 1955. Studies on the fine structure of the mammalian testis. I. Differentiation of the spermatids in the cat (Felis domestica). J. Biphys. Biochem. Cytol. 1(4):287.
- Cobb, L. S. 1967. The fine structure of the pedicellaria of Echinus esculentus (L). II. The sensory system. J. Roy. Microscop. Soc. 88:223.
- Davis, L. E. 1969. Differentiation of neurosensory cells in Hydra. J. Cell Sci. 5:699.
- Davis, L. E. 1974. Ultrastructural studies of the development of nerves in Hydra. Am. Zool. 14:575.
- De The, G. 1964. Cytoplasmic microtubules in different animal cells. J. Cell Biol. 23:265.
- Dewel, W. C. and W. H. Clark, Jr. 1972. An ultrastructural investigation of spermiogenesis and the mature sperm in the anthozoan Bundosoma cavernata (Cnidaria). J. Ultrastruct. Res. 40:417.
- Flock, A. and A. J. Duvall. 1965. The ultrastructure of the kinocilium of the sensory cells in the inner ear and lateral line organs. J. Cell. Biol. 25:1.
- Flock, A. and J. M. Jorgenson. 1974. The ultrastructure of lateral line sense organs in the juvenile salamander Ambystoma mexicanum. Cell Tiss. Res. 152:283.
- Gibbins, I. R., L. G. Tilney and K. R. Porter. 1969. Microtubules in the formation and development of the primary mesenchyme in Arbacia punctulata. I. The distribution of microtubules. J. Cell Biol. 41:201.
- Hinsch, G. W. and W. H. Clark, Jr. 1973. Comparative fine structure of cnidarian spermatozoa. Biol. Reprod. 8:62.
- Hinsch, G. W. 1974. Comparative ultrastructure of cnidarian sperm. Am. Zool. 14:457.
- Horridge, G. A. 1964. Presumed photoreceptive cilia in a ctenophore. Quart. J. Micr. Sci. 105(3):311.

- Karnovsky, M. J. 1965. A formaldehyde glutaraldehyde fixative of high osmolarity for use in electron microscopy. J. Cell Biol. 27:137A.
- Kleve, M. G. and W. H. Clark, Jr. 1974. Fine structure of centriolar specilizations isolated from Hydractinia (Cnidaria) sperm. J. Cell Biol. 63:171A. (Abstract.)
- Miller, R. L. 1973. Chemotaxis of animal sperm. In Behavior of microorganisms. A. P. Miravete, Editor. Plenum Press, London. 31.
- Miller, R. L. 1974. Sperm behavior close to Hydractinia and Ciona eggs. Am. Zool. 14(4):1250. (Abstract.)
- Miller, R. L. and C. Y. Tseng. 1974. Properties and partial purification of the sperm attractant of Tubularia. Am. Zool. 14(2):467.
- Porter, K. R. 1966. Cytoplasmic microtubules and their function. In Principles of Biomolecular Organization. G. E. W. Walstenholme and M. O. Conner, Editors. J. and A. Churchill Ltd., London. 308.
- Rattner, J. B. 1972. Nuclear shaping in marsupial spermatids. J. Ultrastruct. Res. 40:498.
- Reese, T. S. 1965. Olfactory cilia in the frog. J. Cell Biol. 25:209.
- Spurr, A. K. 1969. A low viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26:31.
- Summers, R. G. 1972. A new model for the structure of the centriolar satellite complex in spermatozoa. J. Morphol. 137:229.
- Szollosi, D. 1964. The structure and function of centrioles and their satellites in the jellyfish Phialidium gregarium. J. Cell Biol. 21:465.
- Tilney, L. G. and J. R. Gibbins. 1969. Microtubules in the formation and development of the primary mesenchyme in Arbacia punctulata. II. An experimental analysis of their role in development and maintenance of cell shape. J. Cell Biol. 41:227.
- Tilney, L. G. and J. Goddard. 1970. Nucleating sites for the assembly of cytoplasmic microtubules in the ectodermal cells of blastulae of Arbacia punctulata. J. Cell Biol. 46:564.
- Tokuyosu, U. and E. Yamada. 1959. The fine structure of the retina. Studies with the electron microscope. IV. Morphogenesis of outer segments of retinal rods. J. Biophys. Biochem. Cytol. 6:225.
- Wesall, J., A. Flock and P. G. Lundquist. 1965. Structural basis for directional sensitivity in cochlear and vestibular sensory receptors. Cold Spring Harbor Symp. Quant. Biol. 30:115.
- Westfall, J. A. 1965. Nematocysts of the sea anemone Metridium. Am. Zool. 5:377.
- Westfall, J. A. 1970. The nematocyte complex in a hydromedusan, Gonionemus vertens. Z. Zellforsch. 110:457.